FORM PTO-1390
(REV 12-29-99)

U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER
MBP-005XX

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 36 U.S.C. 371

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)
09/786435

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP99/06433	1 September, 1999	3 September 1998; 16 March 1999

TITLE OF INVENTION: USE OF TGF-BETA INHIBITORS FOR TREATING CEREBRAL DISORDERS

APPLICANT(S) FOR DO/EO/US: Kerstin Kriegelstein

Applicant herewith submits to the United states Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment with the International Preliminary Examination Report mailed December 29, 2000, including amended pages 5-12 and amended claims 1-8.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☒ Other items or information:
    Copy of International Search Report mailed 9 December 1999 for International Application No. PCT/EP99/06433

EXPRESS MAIL NO.
EL634679376US

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/786435	PCT/EP99/06433	MBP-005XX

17. ☒ The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):

		CALCULATIONS PTO USE ONLY
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$1,000.00**	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	**$860.00**	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$710.00**	
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$690.00**	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)	**$100.00**	

ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ N/A	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	13 - 20 =	0	X **$18.00**	$ 0.00	
Independent claims	2 - 3 =	0	X **$80.00**	$ 0.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+**$270.00**	$ N/A	
TOTAL OF ABOVE CALCULATIONS =				$ 860.00	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				$ N/A	
SUBTOTAL =				$ 860.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$ N/A	
TOTAL NATIONAL FEE =				$ 860.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property				$ 0.00	
TOTAL FEES ENCLOSED =				$ 860.00	
				Amount to be Refunded :	$
				Charged:	$

a. ☒ A check in the amount of $860.00 to cover the above fees is enclosed. A check in the amount of $____ is enclosed for the assignment recordation fee.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 23-0804 . A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

Customer Number 207

SEND ALL CORRESPONDENCE TO:

Weingarten, Schurgin, Gagnebin & Hayes LLP
Ten Post Office Square
Boston, Massachusetts 02109

Date: March 2, 2001

SIGNATURE

Charles L. Gagnebin III
NAME

25,467
REGISTRATION NUMBER

CLG/jde/243716

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application	:	Kerstin Kriegelstein
Application No.	:	
Filed	:	Herewith
For	:	USE OF TGF-BETA INHIBITORS FOR TREATING CEREBRAL DISORDERS
Examiner	:	
Attorney's Docket	:	MBP-005XX

Group Art Unit:

* *

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Box PCT, Assistant Commissioner for Patents, Washington, D.C. 20231 on _____.

By: _____
Charles L. Gagnebin III
Registration No. 25,467
Attorney for Applicant

* *

PRELIMINARY AMENDMENT

BOX PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Kindly enter the following Preliminary Amendment in the above-identified application.

IN THE SPECIFICATION:

Please enter amended pages 5-12 of the International Preliminary Examination Report (attached) dated December 29, 2000 for examination.

IN THE AMENDED CLAIMS OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT:

WEINGARTEN, SCHURGIN,
GAGNEBIN & HAYES, LLP
TEL. (617) 542-2290
FAX. (617) 451-0313

Please rewrite Claims 3, 7 and 8 as follows:

3. Use according to claim 1, wherein said disorder is a peripheral and/or CNS-disorder.

7. The pharmaceutical composition according to claim 5, wherein said compound is an antibody directed to TGF-β or a compound having the binding site of a TGF-β receptor.

8. The pharmaceutical composition according to claim 5, wherein said second compound is selected from the group consisting of urokinase and tissue plasminogen activator.

A marked-up version of the above showing additions and deletions is attached for the Examiner's convenience.

Please add new claims 9 through 13 below.

9. Use according to claim 2, wherein said disorder is a peripheral and/or CNS-disorder.

10. Use according to claim 9, wherein said disorder is a cerebral ischemia or a neurodegenerative disorder.

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11. The pharmaceutical composition according to claim 6, wherein said compound is an antibody directed to TGF-β or a compound having the binding site of a TGF-β receptor.

12. The pharmaceutical composition according to claim 6, wherein said second compound is selected from the group consisting of urokinase and tissue plasminogen activator.

13. The pharmaceutical composition according to claim 12, wherein said second compound is selected from the group consisting of urokinase and tissue plasminogen activator.

REMARKS

This Preliminary Amendment puts the claims into proper form for examination. Kindly calculate the filing fee based on the amended claims.

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The Examiner is encouraged to telephone the undersigned attorney to discuss any matter which would expedite allowance of the present application.

Respectfully submitted,

KERSTIN KRIEGELSTEIN

By: _____
Charles L. Gagnebin III
Registration No. 25,467
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Date: 3-2-1

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MARKED-UP VERSION OF AMENDED CLAIMS TO IPER

3. Use according to claim 1~~ or 2~~, wherein said disorder is a peripheral and/or CNS-disorder.

7. The pharmaceutical composition according to claim 5~~ or 6~~, wherein said compound is an antibody directed to TGF-β or a compound having the binding site of a TGF-β receptor.

8. The pharmaceutical composition according to ~~anyone of claims 5 to 7~~ claim 5, wherein said second compound is selected from the group consisting of urokinase and tissue plasminogen activator.

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USE OF TGF-BETA INHIBITORS FOR TREATING CEREBRAL DISORDERS

Description

The present invention relates to the use of a compound capable of substantially inhibiting the biological activity of TGF-β on predamaged neurons, for treating cerebral disorders, and to pharmaceutical compositions containing said compound and a second compound for disintegrating blood clots.

The transforming growth factor β (TGF-β) family contains subspecies TGF-β1, TGF-β2 and TGF-β3, which are widely distributed and contextually acting cytokines with prominent roles in development and cell cycle control (Roberts and Sporn, 1990; Kriegelstein et al., 1995). TGF-βs have been implicated in the regulation of neuronal survival of e.g. motoneurons, sensory and midbrain dopaminergic neurons. The TGF-β isoforms show widely overlapping expression patterns. Nullmutations for each of these isoforms show distinct phenotypes, which, however, are restricted to non-neural tissues (Shull et al., 1992; Kaartinen et al., 1995; Proetzel et al., 1995; Sanford et al., 1997). Mice deficient for the TGF-ß receptor type-II (TβR-II) are lethal at E 10.5, i.e. prior to the development of the nervous system.

During the development of the vertebrate nervous system, large numbers of neurons in the central and peripheral nervous system undergo naturally occuring cell death (Oppenheim, 1991). Regulation of neuron survival or death, respectively, requires the concerted actions of sets of molecules, which act cell-extrinsically and -instrinsically to execute or prevent cell death (Pettmann and Henderson, 1998).

Cerebral disorders such as a neurodegenerative disorder or cerebral ischemiae, result in injury or death of neurons in mammals, and produce motor and/or

cognitive deficits that are often permanent. At present, in most of these cerebral disorders there is no treatment that reliably improves the prognosis of a patient suffering from said disorders.

Thus, the technical problem underlying the present invention is to provide a new system imparting protection and therefore survival on predamaged or injured neurons upon a certain cerebral disorder.

The solution of the above technical problem is achieved by providing the embodiments characterized in the claims.

In particular, the present invention is based on the fact that, beside promoting intact neurons, TGF-β is the major regulator for the execution of predamaged neurons upon a certain cerebral disorder. Accordingly, the present invention relates to the use of a compound capable of substantially inhibiting the biological activity of TGF-β on predamaged neurons, for treating cerebral disorders in mammals, preferably in man. The term "TGF-β" comprises the subspecies TGF-β1, TGF-β2, and TGF-β3.

In a preferred embodiment of the present invention the term "compound capable of substantially inhibiting the biological activity of TGF-β on predamaged neurons" refers to polyclonal or monoclonal antibodies directed to TGF-β, as TGFG-β inhibitors, or to antagonists directed to TGF-β such as compounds having the binding site of a TGF-β receptor, e.g. a TGF-β RII/Fc chimeric protein (TβR-II-Fc), or to proteinaceous or non-proteinaceous compounds which are capable of, e.g. chemically, altering TGF-β in the organism such that the altered TGF-β is rendered incapable of binding to TGF-β receptors, as well as to low molecular weight compounds such as chemical or non-proteinaceous compounds, as TGF-β-antagonists.

The cerebral disorder includes peripheral and/or CNS-disorders including cerebral and focal ischemiae such as apoplexy, and neurodegenerative disorders such as ALS. For example, adverse consequences of central nervous system injuries may

be caused by thrombus, embolus, systemic hypotension, hypertension, hypertensive cerebral vascular disease, rupture of an aneurysm, an angioma, blood dyscrasias, cardiac failure, cardic arrest, cardiogenic shock, septic shock, head trauma, spinal cord trauma, seizure, bleeding from a tumor, or other blood loss. The spinal cord, which is also a part of the central nervous system, is equally susceptible to ischemia resulting from diminished blood flow.

Where the ischemia is associated with a "stroke", it can be either global or focal ischemia, as defined below.

By "focal ischemia", as used herein in reference to the central nervous system, is meant the condition that results from the blockage of a single artery that supplies blood to the brain or spinal cord, resulting in the death of all cellular elements (pan-necrosis) in the territory supplied by that arterty.

By "global ischemia", as used herein in reference to the central nervous system, is meant the condition that results from a general diminution of blood flow to the entire brain, forebrain, or spinal cord, which causes the death of neurons in selectively vulnerable regions throughout these tissues. The pathology in each of these cases is quite different, as are the clinical correlates. Models of focal ischemia apply to patients with focal cerebral infarction, while models of global ischemia are analogous to cardiac arrest, and other causes of systemic hypotension.

A further aspect of the present invention relates to a pharmaceutical composition containing, in pharmaceutically effective amounts, the above defined compound, and a second compound for disintegrating blood clots, and optionally a pharmaceutically acceptable carrier and/or diluent. In a preferred embodiment of the present invention, the second compound is selected from the group consisting of urokinase, thrombin, and tPA (tissue plasminogen activator).

The treatment regimen is carried out, in terms of administration mode, timing of the administration, in dosage, so that the functional recovery of the patient from

the adverse consequence of the cerebral disorder is improved.

The administration of the compound or pharmaceutical composition according to the present invention can be carried out by any standard route and known rule of administration, including intravenously, orally, or intracerebrally. The dosage of such antibodies or antagonists according to the present invention lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized.

The formulation of the above defined compound or the pharmaceutical composition according to the present invention does not exhibit any specific restriction, and may be prepared e.g. in the form of tablets, suppositories, solutions, or retarded release-formulations. The antibodies or antagonists for example can be formulated for parenteral administration by injection, for example, by bolus injection or continuous infusion. Formulations for injection can be presented in unit dosage form, for example, in ampules or in multi-dose containers, with an added preservative.

The therapeutic antibodies or antagonists of the invention can also contain a carrier or excipient and/or diluent, many of which are known to skilled artisans. Excipients which can be used include buffers (for example, citrate buffer, phosphate buffer, acetate buffer, and bicarbonate buffer), amino acids, urea, alcohols, ascorbic acid, phospholipids, proteins (for example, serum albumin), EDTA, sodium chloride, liposomes, mannitol, sorbitol, and glycerol.

It is well known in the medical arts that dosages for any one patient depend on many factors, including the general health, sex, weight, body surface area, and age of the patient, as well as the particular compound to be administered, in the time and route of administration, and other drugs being administered concurrently. Determining the most appropriate dosage and route of administration is well within the abilities of a skilled physician.

ART 34 AMDT

Application No.: PCT/EP99/06433
Applicant: Biopharm Gesellschaft zur biotechnologischen Entwicklung und zum Vertrieb von Pharmaka mbH
"Use of TGF-Inhibitors for treating cerebral disorders"
Our Ref: B 1957 - py / js

New pages 5 to 12 of the description

The following example demonstrates the specifity of anti-TGF-β-antibody as well as morphology and neuron numbers of chick ganglia at E10, i.e. after the main period of ontogenetic ciliary neuron death. Embryos were treated daily from E6 to E9 with 10 μg of a monoclonal mouse anti-TGF-β1, -β2, -β3 antibody (anti-TGF-β; obtained from Genzyme) applied to the chorionic allantoic membrane, an identical dose of a mouse IgG with or without rhodamine conjugation, or 2 μg of a recombinant human TGF-β RII/Fc chimeric protein (TβR-II-Fc; obtained form R&D Systems). Ciliary ganglia were dissected at E10, fixed in Bouin's solution, and paraffin-embedded. Dot blots were prepared showing the specifity of the anti-TGF-β-antibody. Furthermore, sections (H.E. stain or fluorescence microscopy) through the largest circumference of ganglia from embryos were prepared and neuron counts in ganglia with the treatments according to the present invention displayed increased neuron numbers as compared to untreated embryos.

Furthermore, neuron numbers and apoptosis in ciliary ganglia (CG), dorsal root ganglia (L3; DRG), and the lumbar motoneuron column of embryos treated with anti-TGF-β were examined. Neuron counts at E10 following daily treatments from E6 - E9 and TUNEL labelings of CG, DRG, and lumbar motoneurons at E8 following treatment with anti-TGF-β-antibody at E6 and E7 were compared to untreated embryos. A quantitative analysis of TUNEL-positive neurons demonstrated increased or decreased neuron numbers as compared to untreated embryos.

Neuron counts showed a phenotype rescue of CG and motoneurons in anti-TGF-ß-treated embryos by administration of TGF-ß. A treatment with TGF-ß3

(2μg) at E10 following administration of anti-TGF-ß from E6 to E9 produced neuron numbers at E14 identical to control embryos. Continuing treatment with anti-TGF-ß from E10 to E 13 fully maintained neuron numbers.

Qualitative microscopic and quantitative analyses of H.E. stained sections showed that anti-TGF-ß rescues lumbar DRG and motoneurons following unilateral limb bud ablation.

The present invention is illustrated by the following non-limiting example.

EXAMPLE

Neuron counting experiments

Chick embryos were treated daily with 50 μl (phosphate buffered saline, supplemented with 200 units/ml penicillin, 100 μg/ml streptomycin, 200 μg/ml neomycin (each obtained from Gibco) containing either 10 μg of a pan anti-TGF-β (obtained from Genzyme) or 2 μg TβR-II-Fc (obtained from R&D Systems), or 10 μg mouse IgG (obtained from Sigma) by administration onto the chorioallantoic membrane through a window in the shell as described in Oppenheim et al., 1993. Embryos were killed on either E8, E10 or E14. The CG and the lumbar spinal cord with attached DRG were dissected, fixed in Bouin's solution and paraffin embedded. All tissues were sectioned at 8 to 10 μm and stained with H.E. Neurons were counted in every fifth or tenth section as described in Oppenheim et al., 1993.

TUNEL labeling

Sections were stained according to the manufacturer's instructions (Boehringer Mannheim) and counter-stained with H.E. TUNEL positive nuclei of large neurons were counted on every tenth section and expressed as ratio of total neuron of this particular neuron population.

Unilateral limb bud ablation

Ablations of hind limb buds of chick embryos were performed at E3. Embryos were treated daily (10 μg; E3 - E6) as indicated above with either anti-TGF-ß or IgG and processed at E7 for H.E. staining and neuron counting.

Anti-TGF-β-antibody neutralizes endogenous TGF-β during the main period of ontogenetic neuron cell death

In the developing nervous system of chick and mammals, TGF-ß2 and -ß3 as well as their receptors occur in many populations of postmitotic neurons, including CG, DRG, and motoneurons (Krieglstein et al., 1998). A monoclonal antibody recognizing all three isoforms of TGF-ß (anti-TGF-ß) was used to neutralize endogenous TGF-ß during the main period of ontogenetic cell death (E6 - E9) of CG and DRG as well as spinal motoneurons. Ten μg of this antibody neutralize 10 ng of each available recombinant TGF-ß including chicken TGF-ß3 (R&D Systems) to >98%, as assessed in a bioassay using mink lung epithelial cells (Abe et al., 1994; Krieglstein and Unsicker, 1995). The specifity of this monoclonal anti-TGF-ß antibody was ascertained by dot blot using 40 other growth factors. Daily treatments with anti-TGF-ß resulted in an increased size of CG at E10. A corresponding control IgG, which is rhodamine-conjugated, could be detected throughout the entire embryo. A TßR-II-Fc chimeric protein employed as an alternative tool to capture endogenous TGF-ß likewise increased the size of CG at E10 compared to untreated or IgG-treated embryos. Neuron counts revealed that the increased size of CG is due to a significant increase in neuron numbers. The counted values of approximately 6,000 reflect neuron numbers prior to ontogenetic neuron death at E6 (Landmesser and Pilar, 1974) showing that elimination of TGF-ß signalling prevents the execution of ontogenetic neuron death in the chick CG.

Neutralizing TGF-ß interferes with ontogenetic cell death in sensory and motoneurons

Sensory and motoneurons were analyzed to investigate whether neutralizing TGF-ß also interfered with the development of other neuron populations during the period of ontogenetic neuron death. Neuron counts in the L3 DRG and in the lumbar motoneuron column revealed a significant increase in neuron numbers at E10 following daily antibody treatments from E6 to E9. Numbers of motoneurons in antibody-treated embryos almost matched neuron numbers prior to the onset of ontogenetic neuron death (Hamburger et al., 1975). To determine whether the increase in numbers of neurons upon anti-TGF-ß-treatment resulted from reduced ontogenetic neuron death, TUNEL labelling was performed to stain for apoptotic cells. Counting the numbers of TUNEL-positive cells at E8, which is the peak of cell death for all three neuron populations investigated, revealed a significant reduction of the proportion of apoptotic cells. This shows that endogenous TGF-ß is essential for executing the cell death program in developing neurons.

Ontogenetic neuron death occurs after termination of antibody treatment and substitution of endogenous TGF-β

Furthermore, it was investigated whether ontogenetic neuron death would occur at a later time point following termination of the antibody treatment and substitution of endogenous TGF-β. A single dose of TGF-β3 at E10 after the end of the antibody treatment (E6 -E9) resulted in a significant reduction of neuron numbers in the CG and motoneuron column. Numbers of neurons matched those of control embryos at the end of the main ontogenetic cell death period (E10) as well as embryos at E14. Therefore, TGF-β mediates death of selected neurons, especially those that are destined to die. In contrast, prolongation of the antibody treatment beyond the period of ontogenetic death stabilized neuron numbers at increased levels.

Treatment of limb bud-ablated embryos with anti-TGF-β rescues both motoneurons and DRG neurons

Multiple evidence suggests that the extent of ontogenetic neuron death is crucially regulated by the target (Olek and Edwards, 1978; Pittman and Oppenheim, 1979; Pilar et al., 1980; Thoenen and Edgar, 1985), best exemplified by the classic experiments of V. Hamburger (Hollyday and Hamburger, 1976). Extirpation of the hind limb bud in the E3 chick embryo, i.e. prior to the arrival and synapse formation of motoneuron axons in the target (Dahm and Landmesser, 1988, 1991), causes death of almost all lumbar motoneurons and sensory neurons by E7 (Oppenheim et al., 1978). Qualitative microscopic analysis documented that treatment of limb bud-ablated embryos with anti-TGF-ß rescues both motoneurons as well as DRG neurons. Quantification reveals that the lumbar motoneuron population in limb-ablated embryos could surprisingly be rescued by anti-TGF-ß treatment to approximately half the half the size as compared to the non-operated control side.

Summary

The above results show that TGF-ß exerts a key role both in the regulation of ontogenetic neuron death of three major classes of peripheral and CNS neurons as well as in the regulation of cell death following target ablation. Parasympathetic CG, sensory DRG, and spinal lumbar motoneurons in chick embroys have their main period of ontogenetic neuron death between E6 and E9, loose approximately 50% of the total cells generated, but require largely distinct and only partly overlapping target-derived trophic molecules to secure the survival of optimal cell numbers. CG neurons are maintained by CNTF/GPA, DRG neurons mainly by neurotrophins, and motoneurons by GDNF, HGF, neurotrophins, IGF-I and others (Nishi, 1994; Heller et al., 1995; Lewin and Barde, 1996; Oppenheim, 1996). In motoneurons, even a cocktail of numerous trophic molecules cannot fully maintain the population. The example of the present invention demonstrates that the elimination of endogenous TGF-β or TGF-β signaling, respectively, results in rescuing effects, which are distinct from neurotrophic

factor treatments in terms of (i) the broad spectrum of responsive populations and (ii) magnitude of effect, which encompasses virtually all neurons of a given population. Therefore, the lack of TGF-β is able to rescue all neurons destined to die. The elimination of TGF-β is superior to any of known manipulations of the molecular cascade involved in neuron cell death in that neuron populations are fully maintained.

The example shows that ontogenetic neuron death of ciliary, dorsal root and spinal motoneurons is largely prevented and neuron losses following limb bud ablation are greatly reduced following neutralization of endogenous TGF-β by an anti-TGF-β antibody in chick embryos. Likewise, preventing TGF-β signaling by treatment with a TβR-II fusion protein during the period of ontogenetic cell death in the ciliary ganglion rescues all neurons, which normally die. TUNEL staining revealed decreased numbers of apoptotic cells. Application of exogenous TGF-β rescued the deprived phenotype. Thus, TGF-β in contrast to any single neurotrophic factor acting in the above systems plays a key role in regulating ontogenetic neuron death as well as cell death following neuronal target deprivation.

The above results demonstrate the pivotal role of TGF-β in regulating neuron survival and death. The data given above show that TGF-β is an essential trigger of neuronal death and thus, compounds according to the present invention which are capable of inhibiting the biological activity of TGF-β as e.g. TGF-β-specific antibodies are exceptionally good therapeutic tools that prevent TGF-β signalling in the treatment of stroke, neurotrauma, and neurodegenerative diseases.

References

Abe et al., *Anal. Biochem. 216,* 276 (1994)

Dahm and Landmesser, *Dev. Biol. 130,* 621 (1988)

Dahm and Landmesser, *J. Neurosci. 11,* 238 (1991)

Flanders et al., *Development 113,* 183 (1991)

Hamburger, *J. Comp. Neurol. 160,* 535 (1975)

Heller et al., *Development 121,* 2681 (1995)

Hollyday and Hamburger, *J. Comp. Neurol. 170,* 311 (1976)

Kaartinen et al., *Nat. Genet. 11,* 415 (1995)

Krieglstein et al., *Int. J. Dev. Neurosci. 13,* 301 (1995)

Krieglstein and Unsicker, *J. Neurochem. 65,* 1423 (1995)

Krieglstein et al., *J. Neurosci. 18,* 9822 (1998)

Krieglstein et al., *J. Neurobiol. 37,* .563 (1998)

Landmesser and Pilar, *J. Physiol. 247,* 715 (1974)

Lewin and Barde, *Annu. Rev. Neurosci. 19,* 289 (1996)

Nishi, *J. Neurobiol. 25,* 612 (1994)

Olek and Edwards, *Brain Res. 191,* 483 (1978)

Oppenheim et al., *J. Comp. Neurol. 177,* 87 (1978)

Oppenheim, *Annu. Rev. Neurosci. 14,* 453 (1991)

Oppenheim et al., *J. Neurobiol. 24,* 1065 (1993)

Oppenheim, *Neuron 17,* 195 (1996)

Pettmann and Henderson, *Neuron 20,* 633 (1998)

Pilar et al., *J. Neurophysiol. 43,* 233 (1980)

Pittman and Oppenheim, 1979

Proetzel et al., *Nat. Genet. 11,* 409 (1995)

Roberts and Sporn, In: *Handbook of Experimental Pharmacology*, M.B. Sporn and A.B. Roberts, eds. (Springer, Heidelberg), Vol. 95, pp. 419-472 (1990)

Sanford et al., *Development 124,* 2659 (1997)

Shull et al., *Nature 359,* 693 (1992)

Application No.: PCT/EP99/06433
Applicant: Biopharm Gesellschaft zur biotechnologischen Entwicklung und zum Vertrieb von Pharmaka mbH
"Use of TGF-Inhibitors for treating cerebral disorders"
Our Ref: B 1957 - py / js

Claims

1. Use of a compound capable of inhibiting the biological activity of TGF-β on predamaged neurons, for the preparation of a medicament for treating cerebral disorders.

2. Use according to claim 1, wherein said compound is an antibody or an antagonist directed to TGF-β.

3. Use according to claim 1 or 2, wherein said disorder is a peripheral and/or CNS-disorder.

4. Use according to claim 3, wherein said disorder is a cerebral ischemia or a neurodegenerative disorder.

5. A pharmaceutical composition containing, in pharmaceutically effective amounts, a compound capable of inhibiting the biological activity of TGF-β on predamaged neurons, and a second compound for disintegrating blood clots.

6. The pharmaceutical composition according to claim 5 further containing a pharmaceutically acceptable carrier and/or diluent.

7. The pharmaceutical composition according to claim 5 or 6, wherein said compound is an antibody directed to TGF-β or a compound having the binding site of a TGF-β receptor.

8. The pharmaceutical composition according to anyone of claims 5 to 7, wherein said second compound is selected from the group consisting of urokinase and tissue plasminogen activator.

DECLARATION AND POWER OF ATTORNEY

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: USE OF TGF-BETA INHIBITORS FOR TREATING CEREBRAL DISORDERS

the specification of which (check one):

[ ] is attached hereto. [X] was filed 3/2/2001 as Application No. 09/786,435 amended on _____ (if applicable).

[X] was filed as PCT International Application No. PCT/EP99/06433 on September 1, 1999, and was amended under PCT Article 19 on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations §1.56(a).

I hereby claim foreign priority benefits under Title 35, USC §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)		Date Filed	Priority Claimed	
98116692.9	European Patent	3 September 1998	[X]	[ ]
(Number)	(Country)	(Day/Month/Year)	Yes	No
99104033.8	European Patent	16 March 1999	[X]	[ ]
(Number)	(Country)	(Day/Month/Year)	Yes	No

I hereby claim the benefit under Title 35, USC §119(e) of any United States provisional application(s) listed below:

(Application Number)	(Filing Date)
(Application Number)	(Filing Date)
(Application Number)	(Filing Date)
(Application Number)	(Filing Date)

Express Mail Number

EL634464865US

Attorney
Docket No.: MBP-005XX

I hereby claim the benefit under Title 35 USC §120 of any United States application(s) listed below and insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35 USC §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application No.)	(Filing Date)	(Patented/pending/abandoned)
(Application No.)	(Filing Date)	(Patented/pending/abandoned)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) to prosecute this application and transact all business connected therewith in the Patent and Trademark Office, and to file with the USRO any International Application based thereon.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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